

# Cadmium in Blood and Urine—Impact of Sex, Age, Dietary Intake, Iron Status, and Former Smoking—Association of Renal Effects

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We studied determinants of cadmium status and kidney function in nonsmoking men and women living on farms in southern Sweden. Median blood Cd (BCd) was 1.8 nmol/L (range, 0.38–18) and median urinary Cd (UCd) was 0.23 nmol/mmol creatinine (range, 0.065–0.99). The intake of Cd per kilogram body weight did not significantly differ between sexes and did not correlate with BCd or UCd, which may be explained by a low and varying bioavailability of Cd from food items. However, when a subgroup of the study population, couples of never-smoking men and women, were compared, a lower intake per kilogram body weight was found in the women, but the women had a 1.8 times higher BCd and a 1.4 times higher UCd. The higher female BCd and UCd may be explained by higher absorption due to low iron status. BCd and UCd both increased with age and were higher in the ex-smokers, who had stopped smoking more than 5 years before the study, compared to never-smokers. The contribution of locally produced food to the total Cd intake was relatively low and varied. Males living in areas with low soil Cd had lower UCd than the others. However, Cd levels in kidneys from pigs, fed locally produced cereals, did not predict BCd or UCd in humans at the same farms. The kidney function parameter  $\beta_2$ -microglobulin-creatinine clearance was related to UCd, whereas urinary protein-HC, *N*-acetyl- $\beta$ -glucosaminidase or albumin-creatinine clearance was not when age was accounted for. Hence, even at the low exposure levels in this study population, there was an indication of effect on biochemical markers of renal function. **Key words:**  $\alpha_1$ -microglobulin,  $\beta_2$ -microglobulin, kidney, *N*-acetyl- $\beta$ -glucosaminidase, protein-HC, serum ferritin. *Environ Health Perspect* 110:1185–1190 (2002). [Online 30 September 2002]

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Industrialization and increased productivity in agriculture during the last century have caused levels of the toxic metal cadmium in the environment to increase (Andersson and Bingenfors 1985; Petersson-Grawe et al. 1997). Locally, the bedrock and soil may contain naturally high concentrations of Cd; application of Cd-containing phosphate fertilizers and atmospheric deposition further increase the levels. Cd is easily taken up by plants via the roots; acidification of the soil makes Cd more available (Thuvander and Oskarsson 1998). Cd accumulates in the human body; the highest concentrations are found in kidney cortex, which is the critical organ for toxic effects [International Programme on Chemical Safety (IPCS) 1992]. The factors affecting the status of Cd are not sufficiently known, considering the environmental impact and individual factors. Subjects living in areas with Cd-polluted soils had elevated urinary Cd (UCd) levels, which were attributed to consumption of locally grown vegetables and contaminated well water (Sartor et al. 1992; Staessen et al. 1994). However, relations between Cd intake through the diet and blood Cd (BCd) and UCd have not been found at low exposures (Berglund et al. 1994; Reeves and Vanderpool 1997).

Current Cd exposure and cumulative Cd retention can be assessed by measuring Cd in blood and urine, respectively (IPCS 1992).

There are indications that low iron status is linked to increased intestinal absorption of Cd (Flanagan et al. 1978) and increased BCd levels (Berglund et al. 1994), which may explain why women have higher BCd and UCd than men (Buchet et al. 1990; Jawaid et al. 1983). Hence, females may be an at-risk group. Evidence of significant sex differences in BCd levels of nonsmokers are, however, disparate in different countries (Vahter 1982). Current smoking and former smoking habits are important determinants of BCd and UCd (Sartor et al. 1992).

An early sign of Cd-induced kidney dysfunction is urinary excretion of low molecular weight proteins (IPCS 1992). Tubular proteinuria has been shown at UCd concentrations that occur not only in occupationally but also in environmentally exposed European populations (Järup et al. 2000). This important health effect needs to be explored further.

In the present investigation, we studied men and women living at farms in southern Sweden. This is an area with naturally relatively high Cd levels in the soil, as well as atmospheric deposition of Cd from central Europe and intensive farming (Bergbäck and Lohm 1994). The aim was to characterize the Cd status and some of its determinants, as well as kidney function, in couples living at the same farm, consuming locally produced foods, and having presumably similar Cd exposure.

Furthermore, we investigated possible associations between human Cd exposure and Cd concentrations in kidneys from pigs produced on the same farms and fed locally produced cereals.

## Materials and Methods

**Recruitment.** We randomly selected 800 addresses from a Statistics Sweden (SCB) database of approximately 2,400 pig producers in the province of Skåne in southern Sweden. A first questionnaire about the farm and the smoking and dietary habits of the residents was sent to the farmers. Subjects returned 465 (58%) questionnaires with complete answers, of which 224 (48%) volunteered to participate in a more detailed study. From this group, we selected 51 farms. The selection criteria were *a*) production of more than 50% of the pig feed at the farm, *b*) both man and woman at the farm were willing to participate, and *c*) both were nonsmokers. Two farms dropped out during the sampling period. The farms were divided into four geographical groups, as differences in Cd levels could be expected between these areas based on differences in geology and dominating pattern of atmospheric deposition in the region.

**Study population.** One person with glomerular nephritis was excluded from the study, as were 3 persons who were smokers, leaving a total of 105 persons who answered a second detailed questionnaire and volunteered for blood and urine sampling (Table 1). Of these persons, sampled at the 49 farms, 48 were women and 57 were men; 43 (*n* = 86) were married couples, 7 were married

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men (spouse did not volunteer for sampling), 3 were married women, 3 were single women, and 6 were single men. The households had an average of 2.2 (range 1–4) adults. Of the women and men, 55% and 23%, respectively, were also employed outside the farm. The study was approved by the Research Ethics Committee at the University of Lund.

**Questionnaire.** The second food frequency questionnaire (FFQ) was designed to estimate the total Cd intake, the contribution of Cd from different food sources, occurrence of occupational Cd exposure, use of vitamin–mineral supplements (vitaminerals), current health status, and previous smoking habits (number of cigarettes per day, years as a smoker, and years since stopped smoking). The consumption of the different food items was given as frequency and number of pieces or volume of the food and was transformed into weights [Swedish National Food Administration (SLV) 1999]. Information on the amount of water consumed, in the form of drinking water, tea, coffee, and juice, from the respective farm's well was collected. We calculated the intake from water using the analyzed Cd level in water from each farm. The intake from tea, coffee, and juice was estimated from data reported by Jorhem et al. (1984), and used for calculation of the total intake. The Cd concentration of different foods was extracted from the literature, using Swedish data (Jorhem and Sundström 1993, 1995; Jorhem et al. 1984, 1994), except for coarse-grained, whole-meal rye bread and butter, for which German data were used (Müller et al. 1996). Twenty-eight persons reported use of vitaminerals. In the questionnaire 11 women and 7 men reported some physical symptoms [women: cough (1), gastrointestinal problem (2), allergy/asthma (4), high blood pressure (2), migraine (2); men: diabetes (1), muscle and/or joint diseases (3), chronic bowel disease (1), high blood pressure (1), coagulation disorder (1)].

**Sampling.** Food questionnaires and bottles for urine sampling were sent to each farm. Morning urine was collected in acid-washed 250-mL polyethylene bottles with screw caps. All the farms were visited once during a 6-week period in August–October 1998 for collection of food questionnaires and blood and urine samples. Freshly voided urine samples were collected at the time of blood sampling in acid-washed 10-mL polypropylene tubes. Blood samples were collected from the cubital vein in three 10-mL tubes: one serum, one EDTA-prepared (Vacutainer, Becton Dickinson, Rutherford, NJ, USA) and one heparinized (Venoject, Terumo Corp., Tokyo, Japan), which had been tested and found not to release any detectable amounts of Cd. We sampled drinking water from the kitchen tap in a 250-mL polyethylene bottle after flushing water

for 1 min. Sampling from pig feed, feed components, and 421 pigs have been described in detail elsewhere (Lindén 2002).

**Cd in urine, blood, and water.** Blood and urine samples for elemental analysis were frozen (–20°C) until analysis. Blood and urine were diluted 10 times with reagent solution according to Barany et al. (1997). To 2 mL water, 50 µL concentrated nitric acid was added. Internal standards (indium and bismuth 10 µg/L; gallium 20 µg/L; AccuStandard Inc., New Haven, CT, USA) were added. All samples were prepared in duplicate.

We used an inductively coupled plasma mass spectrometry (ICP-MS) instrument (Thermo Elemental, Winsford, Cheshire, UK), with an autosampler (Gilson 222, Gilson, Villiers, France) for analysis, as described by Barany et al. (1997). Interference corrections were made for  $^{114}\text{Cd}$  (corrected for spectral overlap from tin, measured at 118 m/z). Detection limits were calculated (3 times the SD for the reagent blanks); the detection limit was 0.062 nmol/L for water ( $n = 47$ ), 0.53 nmol/L for blood ( $n = 20$ ), and 0.39 nmol/L for urine ( $n = 25$ ).

We checked the accuracies of the analyses against reference materials. For blood analysis, the Seronorm Trace Element Human Whole Blood (Nycomed AS, Oslo, Norway) was used. For batch 404107 ( $n = 4$ ), the analyzed level was  $7.1 \pm 0.6$  (6.4–7.9) nmol/L [mean  $\pm$  SD (range); recommended 6.2 (range 6.0–6.8) nmol/L, with a relative standard deviation (RSD) of 9.1%], and for batch 404108 ( $n = 4$ ),  $54.9 \pm 0.6$  (54–55) nmol/L [recommended 57 (range 56–70) nmol/L; RSD 1.1%]. For urine, Seronorm batch 403125 ( $n = 4$ ), the level was  $42.1 \pm 1.1$  (41–43) nmol/L (recommended 44 nmol/L, RSD 2.4%). For water analysis, the Riverine water reference material for trace elements (SLRS-2; National Research Council, Division of Chemistry, Ottawa, Ontario, Canada) was used ( $n = 11$ ). The analyzed level was  $0.27 \pm 0.03$  (0.27–0.36) nmol/L (certified value 0.25 nmol/L; RSD 9.7%).

**Cd in pig kidney and soil.** The methodology (microwave digestion and graphite furnace atomic absorption spectrometry) for Cd in pig kidney has been described in detail by Olsson and Oskarsson (2001). Details of sampling, analytical precision, and accuracy

have been described in detail by Lindén et al. (In press). Cd levels in soils were interpolated values from a mapping with 338 samples of the arable soils (5,000 km<sup>2</sup>) in Skåne (Eriksson et al. 1995).

**Iron status and kidney function parameters.** The urine samples to be analyzed for  $\beta_2$ -microglobulin (U- $\beta_2$ ) were adjusted to pH 6 with 0.5 mol/L sodium hydroxide. The analysis of blood hemoglobin (Hb) was performed with Sysmex SE 9000 (TOA Medilca Electronics, GmbH, Hamburg, Germany). Automated analyses were used for analysis of serum iron (S-Fe), serum total iron binding capacity (TIBC), serum albumin (S-Alb), serum creatinine (S-creatinine), urinary albumin (U-Alb), and urinary creatinine (U-creatinine) (Hitachi-Modular; Roche Diagnostics GmbH, Mannheim, Germany), and serum ferritin (S-ferritin; AutoDELFIA, Wallac Oy., Turku, Finland). Urinary-N-acetyl- $\beta$ -glucosaminidase (NAG) was analyzed by spectrophotometry, according to Hultberg and Wieslander (1982). Urinary-protein HC (pHC, synonymous to  $\alpha_1$ -microglobulin) was analyzed with a method using polyclonal antibodies (DAKO A/S, Glostrup, Denmark) and the Mancini technique (Järup et al. 2000). The  $\beta_2$ -microglobulin in blood and urine was analyzed with a  $\beta_2$ -micro RIA kit (lot no. AP 3L) and controlled with a  $\beta_2$ -micro control (lot no. AM 2A; Pharmacia Diagnostic AB, Uppsala, Sweden). The  $\beta_2$ -microglobulin detection limit was 0.7 mg/L, and the precision was 16% (RSD in 46 duplicate samples). All urinary parameters were adjusted for creatinine. From S-Alb and S-creatinine, U-Alb and U-creatinine, an albumin-creatinine clearance was calculated. From S- $\beta_2$ , S-creatinine, U- $\beta_2$  and U-creatinine, a  $\beta_2$ -microglobulin-creatinine clearance was calculated.

**Statistics.** We tested data for normality by Kolmogorov-Smirnoff and for the homogeneity of variances by Bartlett's test. Data were log-transformed when necessary. When results were below the formal detection limits for Cd in blood ( $n = 1$ ) and water ( $n = 18$ ), the measured values were used, so that distributions and mean values would not be distorted. Statistical evaluation was performed by analysis of variance (ANOVA), Spearman's rank correlation ( $r_s$ ), and simple and stepwise multiple

**Table 1.** Description of study population ( $n = 105$ ).

	Females		Males	
	Never-smokers	Ex-smokers	Never-smokers	Ex-smokers
No.	38	10	41	16
Age (years)	47.1 $\pm$ 13.6 (14–70)	47.5 $\pm$ 9.8 (34–61)	43.4 $\pm$ 13.1 (16–68)	56.3 $\pm$ 10.8* (37–73)
Weight (kg)	69 $\pm$ 15** (54–113)	75 $\pm$ 12 (54–100)	79 $\pm$ 9 (63–100)	84 $\pm$ 9 (70–105)
BMI (kg/m <sup>2</sup> )	25 $\pm$ 4.3 (19–36)	26 $\pm$ 3.2 (22–32)	24 $\pm$ 2.4 (19–29)	26 $\pm$ 3.4 (20–35)
Lived at the farm (years)	22 $\pm$ 16 (0.5–67)	21 $\pm$ 8.3 (5–31)	33 $\pm$ 13* (8–64)	41 $\pm$ 15* (17–71)

BMI, body mass index. Values shown are mean  $\pm$  SD (range).

\*Significantly different from male never-smokers ( $p = 0.02$ ). \*\*Female never-smokers were significantly less heavy than the male groups ( $p < 0.0001$ ). \*Men had lived longer on the farms than the females ( $p < 0.0001$ ).

linear regression analyses. Post-hoc testing was performed with Games-Howell. The level of significance was set to  $p \leq 0.05$  (two tailed).

## Results

**Cadmium in blood and urine in relation to sex, age, smoking, and vitaminerals.** Cd levels in blood and urine for the whole study group are given in Table 2; iron status and kidney function parameters are given in Table 3. BCd and UCd were positively correlated ( $r_s = 0.64$ ,  $p < 0.0001$ ; Figure 1, Table 4). Women had significantly higher median BCd than men (2.6 and 1.6 nmol/L, respectively; logBCd, ANOVA,  $p = 0.0010$ ), as was also the case for UCd (women, 0.27 and men, 0.19 nmol/mmol creatinine; logUCd, ANOVA,  $p < 0.0001$ ). Age was associated with both BCd and UCd (Table 4). BCd and age were more closely correlated in males than in females.

Of the 105 participants, 79 were never-smokers and 26 were ex-smokers (Table 1). The ex-smokers had smoked on average 16 years, ranging from 1 to 40 years. Between 1 and 46 years (mean 18 years) had elapsed since they stopped smoking; for all but two persons, more than 5 years had elapsed. The total amount smoked (pack-years; packs of cigarettes smoked per day  $\times$  number of years

of smoking) varied between 0.4 and 40 (mean 9.4). There was no statistically significant difference in pack-years between female and male ex-smokers, nor was any relationship found between pack-years and BCd or UCd. Ex-smokers had higher BCd and UCd levels than never-smokers (2.6 vs. 1.6 nmol/L, and 0.25 vs. 0.20 nmol/mmol creatinine, respectively).

The participants using vitaminerals had a median BCd level of 2.5 nmol/L (range 1.1–18 nmol/L) and UCd 0.26 nmol/mmol (0.10–0.99 nmol/mmol) creatinine. For those not using vitaminerals, BCd was 1.7 nmol/L (0.38–7.6 nmol/L) and UCd 0.20 nmol/mmol (0.065–0.70 nmol/mmol) creatinine. Multiple ANOVA analysis including sex, former smoking (yes/no), and use of vitaminerals (yes/no) showed that all three were statistically significantly associated with BCd levels ( $p = 0.0004$ , 0.006, and 0.05, respectively). However, if age was included, vitamineral use was no longer significant. For UCd, only sex and former smoking were statistically significant ( $p < 0.0001$  and  $p = 0.02$ , respectively). Users of vitaminerals had a higher S-ferritin level (96  $\mu\text{g/L}$ ; 4–440  $\mu\text{g/L}$ ) than nonusers (79  $\mu\text{g/L}$ ; 3–277  $\mu\text{g/L}$ ) although the difference was not statistically significant.

**Table 2.** BCd and UCd.

	Total ( <i>n</i> = 105)	Females		Males	
		Never-smokers ( <i>n</i> = 38)	Ex-smokers ( <i>n</i> = 10)	Never-smokers ( <i>n</i> = 41)	Ex-smokers ( <i>n</i> = 16)
BCd <sup>a</sup> (nmol/L)	2.3 $\pm$ 1.9 1.8 (0.38–18)	2.6 $\pm$ 1.4 2.3 (0.66–5.7)	3.4 $\pm$ 1.8 3.1 (1.4–7.6)	1.9 $\pm$ 2.6* 1.4 (0.38–18)	2.2 $\pm$ 0.68 2.0 (1.0–3.5)
UCd <sup>b</sup> (nmol/mmol creatinine)	0.26 $\pm$ 0.15 0.23 (0.065–0.99)	0.30 $\pm$ 0.17 0.26 (0.097–0.99)	0.40 $\pm$ 0.17 0.35 (0.23–0.70)	0.18 $\pm$ 0.08* 0.17 (0.065–0.41)	0.26 $\pm$ 0.13 0.24 (0.15–0.66)

Values shown are mean  $\pm$  SD and median (range).

<sup>a</sup>Conversion factor: 1 nmol/L = 0.112  $\mu\text{g/L}$ . <sup>b</sup>Conversion factor: 1 nmol Cd/mmol creatinine = 0.994  $\mu\text{g Cd/g creatinine}$ .

\*Significantly lower than the male ex-smokers and both female groups ( $p < 0.0001$ ).

**Table 3.** Iron status and kidney function parameters.

Parameter	Females		Males	
	Nonsmokers ( <i>n</i> = 38)	Ex-smokers ( <i>n</i> = 10)	Nonsmokers ( <i>n</i> = 40)	Ex-smokers ( <i>n</i> = 17)
Hb (g/L)	134 $\pm$ 11* 136 (97–148)	134 $\pm$ 6* 132 (128–148)	145 $\pm$ 10 146 (118–164)	144 $\pm$ 8 144 (126–157)
S-Fe ( $\mu\text{mol/L}$ )	16 $\pm$ 6 16 (3–29)	16 $\pm$ 5 16 (7–24)	17 $\pm$ 6 16 (2–28)	16 $\pm$ 4 16 (8–24)
TIBC ( $\mu\text{mol/L}$ )	70 $\pm$ 13** 66 (49–102)	72 $\pm$ 12 73 (56–91)	64 $\pm$ 7 66 (49–78)	60 $\pm$ 8** 64 (46–71)
S-Ferritin ( $\mu\text{g/L}$ )	46 $\pm$ 33* 41 (3–117)	41 $\pm$ 24* 40 (8–84)	110 $\pm$ 59 101 (17–302)	128 $\pm$ 97 99 (35–440)
$\beta_2$ -creatinine clearance (%)	0.13 $\pm$ 0.15 0.057 (0.028–0.83)	0.089 $\pm$ 0.067 0.065 (0.025–0.20)	0.066 $\pm$ 0.038 0.056 (0.020–0.18)	0.086 $\pm$ 0.070 0.069 (0.020–0.30)
pHC (mg/mmol creatinine)	0.54 $\pm$ 0.43 0.40 (0.20–2.5)	0.43 $\pm$ 0.21 0.41 (0.16–0.76)	0.52 $\pm$ 0.30 0.50 (0.06–1.6)	0.74 $\pm$ 0.39 0.66 (0.23–1.7)
NAG (units/mmol creatinine)	0.19 $\pm$ 0.19 0.16 (0.01–1.2)	0.16 $\pm$ 0.09 0.13 (0.03–0.34)	0.14 $\pm$ 0.07 0.13 (0.02–0.36)	0.22 $\pm$ 0.22 0.16 (0.05–1.0)
Albumin-creatinine clearance ( $\times 10^{-6}$ )	2.0 $\pm$ 1.8 1.4 (0.50–9.2)	2.3 $\pm$ 2.4 1.7 (0.33–8.9)	1.6 $\pm$ 2.6 1.1 (0.29–17)	1.5 $\pm$ 1.2 1.0 (0.40–4.2)

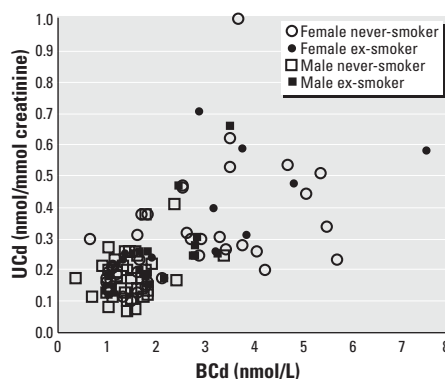
Values shown are mean  $\pm$  SD and median (range).  $\beta_2$ -creatinine clearance = quotient between  $\beta_2$ -microglobulin in urine and serum, adjusted for creatinine. Albumin-creatinine clearance = quotient between albumin in urine and serum, adjusted for creatinine.

\*Significantly lower than the male groups. \*\*Groups significantly different from each other.

**Cd intake.** Cd intake per kilogram body weight per week showed no statistically significant correlation with BCd or UCd, neither in the whole group nor in the never-smokers (Figure 2). Men had a higher total intake than women (ANOVA,  $p < 0.0001$ ), but for the whole study population there was no statistically significant difference when calculated on a body weight basis (Table 5). Women had a higher relative Cd contribution from vegetables, potatoes, and roots than men, while men had a higher Cd contribution from bread than women (data not shown). Out of the weekly consumption, the vegetable food groups contributed 83% of the total Cd intake, although constituting only 29 weight-percent (w%) of all consumed food. Bread was the largest contributor (36%), followed by potatoes and roots (24%), and vegetables (8.4%; Table 5).

For individuals consuming mushrooms ( $n = 81$ ), the Cd contribution was 0.5% (Table 5; range 0.009–9.1%). Most of these individuals consumed the commercially cultivated *Agaricus bisporus* ( $n = 75$ ). In subjects who ate wild mushroom species ( $n = 6$ ), the Cd contributions were 0.8–9.1%. In 18 persons stating intake of offal, its average Cd contribution was 0.3% (range 0.5–3.6%). Only one person reported consumption of kidney. A total of 57 individuals reported eating shrimp, crayfish, and/or crabs, most of them ( $n = 49$ ) only shrimp. Intake of crabs had a high impact on Cd intake, contributing 2.5–53%. The average Cd intake was as high as  $183 \pm 80$   $\mu\text{g/week}$  (105–296  $\mu\text{g/week}$ ) for the crab consumers ( $n = 5$ ). Because there were few crab and crayfish consumers ( $n = 4$ ) and because of the large effect on the total average intake from the crabs, those food items are excluded in Table 5. However, for individual intake figures, the person's actual calculated intake, including all shellfish, was used.

The Cd concentration in drinking water was  $0.020 \pm 0.033$   $\mu\text{g/L}$  (range –0.001 to 0.21  $\mu\text{g/L}$ ;  $n = 49$ ); the water contributed only 0.2% of the total Cd intake (0–1.7%).



**Figure 1.** Relation between BCd and UCd in the total study population ( $r_s = 0.64$ ,  $p < 0.0001$ ). One male (BCd = 18 nmol/L, UCd = 0.27 nmol/mmol creatinine) is not shown in the plot.



Of the study population, 103 persons reported consumption of locally produced food;  $18 \pm 11\%$  (0.5–45%) of the food consumed was local. The average Cd contribution was  $21 \pm 13 \mu\text{g}/\text{week}$  (0.6–50  $\mu\text{g}/\text{week}$ ), which corresponds to 17% of the total Cd intake. Meat and potatoes were the two locally produced food items most commonly consumed. Of these, potatoes contributed the most to the total Cd intake (13  $\mu\text{g}/\text{week}$ ). The individual with the highest consumption of locally produced food (45 w%) had a total intake of 99  $\mu\text{g}/\text{week}$  [ $1.4 \mu\text{g}/\text{kg}$  body weight (bw)/week] with 22  $\mu\text{g}$  from potatoes and 16  $\mu\text{g}$  from milk.

**Intracouple correlations.** A total of 24 never-smoking couples were available for comparison between male and female living at the same farm. A strong correlation was seen for Cd intake ( $r_s = 0.75$ ,  $p = 0.0002$ ) in this subgroup. Paired comparison showed that men (1.68  $\mu\text{g}/\text{kg}$  bw/week) had a higher Cd intake than women (1.50  $\mu\text{g}/\text{kg}$  bw/week;  $p = 0.05$ ). A very close association was seen for age within the couples ( $r_s = 0.97$ ,  $p < 0.001$ ). The couples' age-adjusted BCd and UCd were not significantly correlated. The intracouple female/male ratio showed that women had on average 1.8 (0.09–4.4) times higher BCd and 1.4 (0.63–3.9) times higher UCd than men ( $p = 0.0005$  and  $0.008$ , respectively).

**Geographical differences.** The age-adjusted logBCd and logUCd for never-smokers were analyzed by ANOVA for geographical location. Male UCd in the northeastern area (0.14 nmol/mmol creatinine;  $n = 5$ ) was significantly lower than male UCd in the rest of Skåne (0.21 nmol/mmol creatinine;  $n = 27$ ;  $p = 0.002$ ). For females there were no significant differences. The mean soil levels in the different areas were 0.20, 0.23, 0.35, and 0.25 mg Cd/kg dry matter for the northeast, northwest, southeast, and southwest, respectively. The northeastern and northwestern areas had statistically significantly lower soil Cd levels than the southeast (northeast vs. southeast,  $p = 0.005$ ; northwest vs. southeast,  $p = 0.04$ ).

**BCd and UCd versus Cd levels in pig kidney.** Human BCd and UCd levels were tested

for relationships with pig kidney Cd concentrations [ $n = 421$ ,  $151 \pm 72 \mu\text{g}/\text{kg}$ ; mean  $\pm$  SD (median 134, range 43–680), Lindén et al. In press] from the same farm. One male never-smoker per farm ( $n = 30$ ) was included in the analysis. A significant negative simple regression was found for logUCd versus log pig kidney Cd concentrations ( $r = -0.24$ ,  $p = 0.0003$ ).

**Iron status versus BCd and UCd.** BCd was statistically significantly inversely correlated with S-ferritin, but only in women (Figure 3; Table 4). For none of the other iron parameters were there any significant associations.

**Multivariate analysis of determinants and BCd and UCd.** Determinants [S-ferritin, age, and former smoking (no = 0, yes = 1)] for BCd and UCd were evaluated by stepwise multiple regressions for females and males separately. For BCd, the optimal model for females was  $\log\text{BCd} = 0.11 + 0.010 (\text{age}) - 0.004 (\text{S-ferritin})$  ( $R^2 = 0.36$ ,  $p < 0.0001$ ). For males, the optimal model was  $\log\text{BCd} = -0.13 + 0.007 (\text{age})$  ( $R^2 = 0.19$ ,  $p = 0.0004$ ). The same procedure for UCd resulted in the following model for females:  $\log\text{UCd} = -1.06 + 0.012 (\text{age}) + 0.13 (\text{former smoking}) - 0.002 (\text{S-ferritin})$  ( $R^2 = 0.50$ ,  $p < 0.0001$ ). For males, again, only age was statistically significant:  $\log\text{UCd} = -1.16 + 0.009 (\text{age})$  ( $R^2 = 0.38$ ,  $p < 0.0001$ ).

**Parameters of kidney function.** In the total study population ( $n = 105$ ), there were statistically significant correlations between UCd and all kidney function parameters (Table 4). UCd versus NAG and pHc were statistically significant for both sexes (Table 4). Prediction of kidney function parameters was tested for logUCd and adjusted for age. For albumin-creatinine clearance, the model was not statistically significant. For both pHc and NAG, age was the only significant determinant [ $\log\text{pHc} = -0.72 + 0.008 (\text{age})$ ;  $R^2 = 0.15$ ,  $p < 0.0001$ ;  $\log\text{NAG} = -1.27 + 0.009 (\text{age})$ ,  $R^2 = 0.125$ ,  $p = 0.0001$ , respectively]. However, the  $\beta_2$ -creatinine clearance was explained by the UCd ( $\log\beta_2\text{-creatinine clearance} = -0.94 + 0.35 \log\text{UCd}$ ,  $R^2 = 0.056$ ,  $p = 0.010$ ), while age did not have a significant influence.

**Table 4.** Associations between BCd and UCd versus iron status and kidney function parameters for the total study population ( $n = 105$ ) (Spearman's rank correlation coefficient;  $r_s$ ).

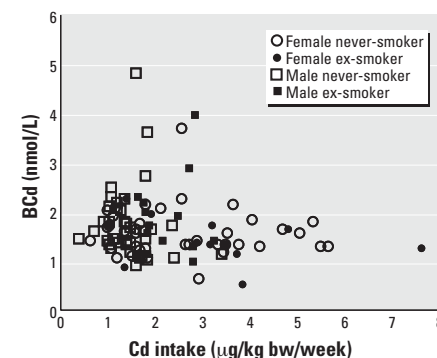
Parameters	Total		Females		Males	
	$r_s$	$p$ -Value	$r_s$	$p$ -Value	$r_s$	$p$ -Value
BCd vs. UCd	0.64	< 0.0001*	0.67	< 0.0001*	0.45	0.0008*
Age	0.41	< 0.0001*	0.33	0.021*	0.50	0.0002*
Hb	-0.22	0.025*	-0.078	0.59	-0.067	0.61
S-ferritin	-0.41	< 0.0001*	-0.44	0.0026*	-0.11	0.39
UCd vs. age	0.58	< 0.0001*	0.64	< 0.0001*	0.66	< 0.0001*
S-ferritin	-0.30	0.003*	-0.10	0.51	$2 \times 10^{-4}$	1.0
$\beta_2$ -creatinine clearance	0.21	0.04*	0.24	0.10	0.16	0.25
NAG	0.28	0.0055*	0.29	0.048*	0.31	0.023*
pHc	0.22	0.024*	0.27	0.065	0.38	0.0052*
Albumin-creatinine clearance	0.22	0.030*	0.37	0.012*	-0.13	0.35

\*Significant correlations.

## Discussion

Women had approximately 1.4 times higher BCd and 1.6 times higher UCd than men. This confirms previous findings in randomly selected persons (Buchet et al. 1990; Jawaid et al. 1983). S-ferritin was shown to have a high impact on BCd, but only in women. The lack of relationship in men in the present study is most probably due to the fact that most of them had normal to high S-ferritin levels. There was no statistically significant difference between men and women in Cd intake per kilogram body weight for the whole study population. However, when comparing the never-smoking couples, women had a lower intake per kilogram body weight than their husbands. Despite this, women had higher BCd and UCd. The sex difference is probably explained by the higher occurrence of low S-ferritin in women, resulting in a higher uptake of Cd, which is in accordance with earlier findings (Berglund et al. 1994), as well as a biokinetic model on UCd (Choudhury et al. 2001). Even when allowing for various determinants (age, sex, former smoking, S-ferritin), there was a large unexplained interindividual variation in BCd and UCd, which may partly be due to genetic factors, as shown for females by Björkman et al. (2000).

BCd and UCd increased with age in both men and women. BCd is considered to reflect current exposure. Ex-smokers, both men and women, had higher Cd levels in blood and urine than never-smokers. All except two had stopped smoking more than 5 years ago; exclusion of these subjects did not change the results. The half-life of Cd in blood is approximately 2–3 months (Welinder et al. 1977); thus elevated levels of BCd because of former smoking might not be expected. BCd levels are, however, also influenced by the body burden of Cd, which is elevated for long periods of time after end of exposure due to the long-term retention of Cd in kidney and liver (Berglund et al. 1994; Hoffmann et al. 2001; Welinder et al. 1977). Thus, former smoking,



**Figure 2.** BCd versus weekly Cd intake per kilogram body weight. No statistically significant correlations were found.

even more than 5 years ago, caused increased BCd, which should be considered in biomonitoring of Cd exposure.

Dietary intake is supposed to be the main source of exposure to Cd in the general non-smoking population. As in the present study, a lack of correlation between BCd or UCd, on the one hand, and Cd intake, on the other, has been reported previously at low exposure levels (Berglund et al. 1994). One reason for the present lack of association may be the low and varying bioavailability of Cd from food items (Chan et al. 2001; Lind et al. 1995, 1998) and/or the uncertainty in estimation of Cd intake. In Japan, with a higher dietary exposure and with rice contributing about 40% of the dietary Cd intake, a correlation has been seen (Shimbo et al. 2000; Watanabe et al. 2000).

In this physically active population group with a food intake between the 75th and 90th percentile compared with the general Swedish population (SLV 1994), the intake of Cd was

1.4–2.0 times higher than previously reported for mixed diets in Sweden (Becker and Kumpulainen 1991; Slorach et al. 1991; Vahter et al. 1990). There may be some overestimation of Cd intake in this study, as older data on Cd levels were used for about half of the food items, with possibly higher Cd levels than the present ones. However, special emphasis in this FFQ was paid to detection of consumption of food items known to have high Cd concentrations. Due to the slow turnover of Cd in the body, an FFQ that reflects long-term food consumption should be relevant. Duplicate-portion studies give a more accurate figure of recent intake but have the disadvantage of only covering a short period of time and usually comprise only a few participants.

In spite of the stratification of the study population, the consumption of locally produced food items varied considerably. The average Cd contribution from locally produced food was not more than 17% of the

total Cd intake; however, the range was wide (0.5–41%). Thus, while in most subjects the impact was limited, for some individuals the local environmental Cd levels should be important. In a Canadian study, a 26% Cd contribution from traditional food of local origin (wild caribou, moose, and fish products), was reported in a population with approximately the same level of estimated Cd intake from the diet (about 120 µg/week) (Kim et al. 1998).

Men living in the northeastern study area had lower UCd levels than those living in other parts of Skåne. The northeastern area has lower levels of Cd in the arable soils, as compared to the other parts (Eriksson et al. 1995); hence there should be some local influence. In accordance with this, Sartor et al. (1992) showed higher UCd excretion in areas with Cd-polluted soils. However, Cd in pig kidneys could not be used to predict Cd concentrations in human blood and urine in the present study, even though cereals are a substantial part of both the human and pig diets. This is probably because the cereals and other foods consumed by the humans were mainly from nonlocal sources. Further, in pig feed, other ingredients than locally produced cereals contributed to a large part of the Cd intake (Lindén et al. 1999, 2001).

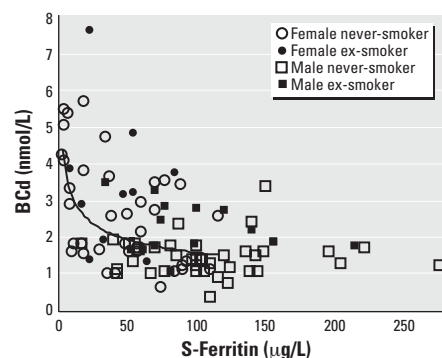
The indication of higher BCd and UCd in persons using vitamin and/or mineral supplements is interesting. Supplementation with iron, at least in persons with low iron status, would be expected to decrease Cd absorption from the diet. Thus, our findings arouse suspicion that mineral supplements (e.g., zinc) might be contaminated with Cd. A high Cd contamination has been shown in vitamin–mineral supplements used for pig feed (Lindén et al. 1999, 2001). Further investigations of human vitamin and mineral supplements would be of interest.

The BCd levels found in this study are in agreement with levels reported during the last

**Table 5.** Cadmium intake [mean ± SD (range)] from different food groups.

Food group	No. <sup>a</sup>	Consumption		Cd intake	
		g/week	w% <sup>b</sup>	µg/week	Percent of total
Bread	105	1,560 ± 45 (270–7,900)	7.9	45 ± 31 (6.0–230)	36
Cereals and rice	104	292 ± 185 (6.4–970)	1.5	8.2 ± 5.7 (0.25–29)	7.0
Seeds and chocolate	43	47 ± 70 (0.0072–400)	0.2	8.9 ± 13 (0.032–60)	2.8
Potatoes and roots	105	1,527 ± 585 (410–4,300)	7.7	28 ± 11 (7.8–78)	24
Vegetables	105	585 ± 504 (50–2,800)	3.0	9.4 ± 8.1 (0.8–45)	8.4
Mushrooms	81	35 ± 28 (2.5–120)	0.2	0.79 ± 1.4 (0.028–7.1)	0.5
Fruits and berries	103	1,420 ± 1,020 (53–6,900)	7.2	4.0 ± 2.8 (0.17–18)	3.5
Fats and oils (vegetable)	98	292 ± 198 (25–1,200)	1.5	0.54 ± 0.39 (0.045–2.2)	0.4
Milk and milk products	105	4,967 ± 3,006 (587–20,000)	25.2	6.7 ± 3.7 (1.2–24)	5.8
Meat, fish, and eggs	105	1,610 ± 668 (600–3,300)	8.1	2.0 ± 0.87 (0.85–5.0)	1.8
Offal	18	39 ± 22 (12.5–100)	0.2	1.8 ± 1.0 (0.56–4.4)	0.3
Shrimp	50	76 ± 91 (8.75–600)	0.4	10 ± 12 (0.24–47)	3.9
Coffee, tea, and juice	104	7,290 ± 4,630 (104–23,000)	36.9	6.0 ± 4.0 (0.10–18)	5.3
Total	105	19,500 ± 6,320 (7,100–43,000)	100	118 ± 39 <sup>c</sup> (38–300)	99.8 <sup>d</sup>
Intake					
Women	48	16,700 ± 5,410 (7,100–33,000)		104 ± 28* (38–210)	
Men	57	21,900 ± 6,120 (10,000–43,000)		136 ± 49 (81–300)	
Cd intake <sup>e</sup> (µg/kg bw/week)					
Women	48	—		1.53 ± 0.51 (0.52–3.7)	
Men	57	—		1.73 ± 0.73 (0.90–4.8)	

<sup>a</sup>Number of persons reporting consumption of food items in this food group at least once a month. <sup>b</sup>w% of total amount of food; water, constituting 36 w%, is excluded in the calculations. <sup>c</sup>Total weekly intake when consumption of crabs and crayfish are excluded. <sup>d</sup>0.2% of the total Cd intake is from water; data shown in "Results." <sup>e</sup>Intake of Cd per kilogram body weight and week. \*Females had a significantly lower intake of Cd per week than men (ANOVA,  $p < 0.0001$ ).



**Figure 3.** BCd versus S-ferritin in the study population. Data from one man (BCd = 18 nmol/L, S-ferritin = 45 µg/L) is not shown in the plot. An adjusted exponential line for female never-smokers is shown ( $y = 5.97x^{-0.29}$ ,  $R^2 = -0.28$ ).

two decades in Sweden (Skerfving et al. 1999) in adult nonsmokers and in adolescents sampled in 1993–1994 (Barany et al. 2002). The UCd is also similar to (Jawaid et al. 1983) or slightly higher (Berglund et al. 1994) than in nonoccupationally exposed nonsmokers in earlier Swedish studies. The mean UCd in the present study was about 25% (men) to 50% (women) of those reported in a Swedish population living close to a former battery plant, including both occupationally and/or environmentally exposed individuals (Järup et al. 2000). The UCd in most of the persons in our study population was < 1 nmol/mmol creatinine, a level at which NAG excretion starts to increase (Nortier et al. 1997). However, despite the low UCd, the kidney function parameter  $\beta_2$ -creatinine clearance was positively related to the Cd levels in the whole study group, even when age was accounted for. Hence, this may indicate an adverse health effect. The causal contribution of Cd to impairment of the other renal function parameters may have been underestimated due to overcontrolling for age, resulting from highly significant correlations between UCd and age. A fairly large proportion of the women (33%) and men (14%) had  $\beta_2$ -creatinine clearance > 0.1%, indicating a slightly decreased reabsorption of  $\beta_2$ -microglobulin in the proximal renal tubules (Järup et al. 2000). The effect is limited; however, on a population basis, an exposure only moderately higher than this might be of clinical significance (Hellström et al. 2001).

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